Genetic Interactions Between *CDC31* and *KAR1*, Two Genes Required for Duplication of the Microtubule Organizing Center in *Saccharomyces cerevisiae*

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ABSTRACT

KAR1 encodes an essential component of the yeast spindle pole body (SPB) that is required for karyogamy and SPB duplication. A temperature-sensitive mutation, *kar1-Δ17*, mapped to a region required for SPB duplication and for localization to the SPB. To identify interacting SPB proteins, we isolated 13 dominant mutations and 3 high copy number plasmids that suppressed the temperature sensitivity of *kar1-Δ17*. Eleven extragenic suppressor mutations mapped to two linkage groups, *DSK1* and *DSK2*. The extragenic suppressors were specific for SPB duplication and did not suppress karyogamy-defective alleles. The major class, *DSK1*, consisted of mutations in *CDC31*. *CDC31* is required for SPB duplication and encodes a calmodulin-like protein that is most closely related to caltractin/centrin, a protein associated with the Chlamydomonas basal body. The high copy number suppressor plasmids contained the wild-type *CDC31* gene. One *CDC31* suppressor allele conferred a temperature-sensitive defect in SPB duplication, which was counter-suppressed by recessive mutations in *KAR1*. In spite of the evidence for a direct interaction, the strongest *CDC31* alleles, as well as both *DSK2* alleles, suppressed a complete deletion of *KAR1*. However, the *CDC31* alleles also made the cell supersensitive to *KAR1* gene dosage, arguing against a simple bypass mechanism of suppression. We propose a model in which Karlp helps localize Cdc31p to the SPB and that Cdc31p then initiates SPB duplication via interaction with a downstream effector.

EUKARYOTIC cells require microtubule-based cytoskeletal arrays for the essential processes of chromosome segregation and organelle movement. The *in vivo* regulation and control of the dynamic microtubule cytoskeleton are not well understood, although the principal structural subunit, tubulin, has been characterized in detail. It is thought that microtubule-associated proteins play critical roles in the mediation of cytoskeletal functions. These proteins are found along the length of the polymer as well as at nucleation sites called microtubule organizing centers (MTOCs). MTOCs are small and complex, which has made them relatively recalcitrant to biochemical analysis, but they should be amenable to genetic approaches. A compelling example of the utility of the genetic approach is the identification of γ-tubulin via suppressor analysis of mutations in the *Aspergillus nidulans* β-tubulin gene (WEIL *et al.* 1986; OAKLEY and OAKLEY 1989). γ-Tubulin is associated with the Aspergillus MTOC where it is required for microtubule assembly (OAKLEY *et al.* 1990). Homologs of γ-tubulin have been found associated with the MTOCs of many eukaryotic organisms (ZHENG *et al.* 1991; STEARNS *et al.* 1991). Antibodies to mammalian γ-tubulin block microtubule nucleation from centrosomes *in vivo* (JOSHI *et al.* 1992).

The yeast, *Saccharomyces cerevisiae*, has a single MTOC called the spindle pole body (SPB). The SPB is embedded in the nuclear envelope and is associated with both nuclear and cytoplasmic microtubules (reviewed by WINEY and BYERS 1992). Mutations that affect the functions of the SPB or its associated microtubules have been isolated in a variety of approaches (for a recent review, see SOLOMON 1991). These include conditional mutations that affect normal progression through the cell cycle, as well as mutations that affect chromosome stability and nuclear fusion. It is not yet clear how many of these genes directly affect SPB or microtubule function.

KAR1 was originally identified by a mutation, *kar1-1*, that affects the frequency of nuclear fusion or karyogamy (CONDE and FINK 1976; FINK and CONDE 1976). Although the *kar1-1* mutation causes no associated mitotic defects, other mutations demonstrated that *KAR1* is required for SPB duplication (ROSE and FINK 1987). The two functions of *KAR1*, karyogamy and SPB duplication, are separable and map to discrete domains of the protein (VALLEN *et al.* 1992b). Hybrid Karl-β-galactosidase proteins containing the region of Karlp required for SPB duplication localize specifically to the SPB, suggesting Karlp itself is associated with the SPB (VALLEN *et al.* 1992a). A small deletion within this "SPB domain" produces a temperature sensitive *kar1* allele, *kar1-Δ17* (VALLEN *et al.* 1992b).

Like mitotically defective *kar1* mutations, temperature-sensitive *cdc31* alleles cause cell cycle arrest with a large bud and a single enlarged SPB (BYERS 1981; WINEY *et al.* 1991). This phenotype suggests that *CDC31* and *KAR1* are required at a similar stage in the SPB duplication pathway. Sequence analysis demonstrated that

TABLE 1

Yeast strains

Strain	Genotype	Source[a]
MS10	*MATa ura3-52 leu2-3 leu2-112 ade2-101*	
MS37	*MATα ura3-52 leu2-3 leu2-112 ade2-101*	
MS52	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1*	
MS53	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1*	
MS142	*MATa trp1-Δ1 lys2-801 ade2-101 cyh2^R*	
MS147	*MATa trp1-Δ1 lys2-801 ade2-101 cyh2^R* [rho^o]	
MS739	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-1*	
MS1113	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ15*	
MS1117	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ13*	
MS1267	*MATa ura3-52 ade2-101 trp1-Δ1 cyh2^R kar1-1*	
MS1269	*MATa ade2-101 trp1-Δ1 lys2-801 cyh2^R kar1-1*	
MS1272	*MATa ura3-52 trp1-Δ1 lys2-801 cyh2^R kar1-Δ15*	
MS1274	*MATa ura3-52 trp1-Δ1 lys2-801 cyh2^R kar1-Δ13*	
MS1554	*MATa ura3-52 leu2-3 leu2-112 ade2-101 his3-Δ200*	
MS1697	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ2* [pMR76: YCp50 *KAR1*]	
MS1699	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ2* [pMR76: YCp50 *KAR1*]	
MS2082	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17*	
MS2083	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17*	
MS2087	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17*	
MS2356	*MATα ura3-52 leu2-3 leu2-112 trp1-Δ1 CDC31*::pMR2000 [pMR2000: *CDC31* on pRS406]	
MS2384	*MATα ura3-52 leu2-3 leu2-112 ade2-101 trp1-Δ1 kar1-Δ18* [pMR76: YCp50 *KAR1*]	
MS2385	*MATa ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ18* [pMR76: YCp50 *KAR1*]	
MS2468	*MATa ura3-52 trp1-Δ1 ade2-101 lys2-801 CDC31-16*[b]	
MS2474	*MATa ura3-52 trp1-Δ1 ade2-101 CDC31-16*[b]	
MS2623	*MATa ura3-52 trp1-Δ1 ade2-101 lys2-801 CDC31-16*[b]	
MS2978	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17-SUP^{10A}*	
MS2979	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17-SUP^{1C1}*	
MS3133	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 DSK2-2*	
MS3134	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 DSK2-1*	
MS3135	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 CDC31-15*	
MS3136	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 CDC31-17*	
MS3137	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 CDC31-16*	
MS3138	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 CDC31-18*	
MS3139	*MATα ura3-52 leu2-3 leu2-112 ade2-101 kar1-Δ17 CDC31-19*	
MS3140	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17 CDC31-11*	
MS3141	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17 CDC31-12*	
MS3142	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17 CDC31-13*	
MS3143	*MATa ura3-52 leu2-3 leu2-112 trp1-Δ1 kar1-Δ17 CDC31-14*	
MS3510	*MATα ura3-52 leu2-3 leu2-112 ade2-101 CDC31-16*	
PB9-33B	*MATα ura3 leu2-3 leu2-112 trp1 his3* or *his7 cdc31-1*	B. Byers
A32-17A	*MATα ura3 leu2 trp1 ade2 cyh2^R cdc31-2*	B. Byers
12-2B-2D	*MATα ura3 cyh2^R met2 ade cdc31-5*	B. Byers
MY768	*MATa his1*	
MY2901	*MATα ura3-52 his4-Δ29*	G. Fink
MY2902	*MATα ura3-52 his4-Δ29 kem1-1*	G. Fink

[a] Unless otherwise noted, all strains are from this study and are isogenic with S288C. MY768 and the strains from B. Byers and G. Fink are not isogenic with S288C.

[b] The *CDC31-16* allele was originally called *DSK1-6*.

CDC31 has homology to the calmodulin family of Ca^{2+}-regulatory proteins (Baum *et al.* 1986). In addition, the protein most closely related to *CDC31*, caltractin/centrin (Huang *et al.* 1988a,b; Salisbury *et al.* 1988), is associated with the basal body in Chlamydomonas. Recently, Cdc31p has been shown to localize to the SPB in yeast (Sprang *et al.* 1993; S. Biggins and M. Rose, manuscript in preparation).

We have isolated and characterized 13 intra- and extragenic temperature-resistant suppressor alleles of the *kar1-Δ17* mutation. Since the region deleted in *kar1-Δ17* is required for SPB localization, suppressors might identify other SPB-associated components or regulators of SPB duplication. Nine of the suppressors isolated in this analysis are alleles of *CDC31*. Based on the analysis of mutations in *CDC31* and *KAR1*, we present possible models for the role of these proteins in SPB duplication. In addition to the *CDC31* alleles, we have identified two alleles in a second genetic locus, *DSK2*, in which dominant mutations suppress *kar1-Δ17* and two intragenic pseudorevertant alleles within *kar1-Δ17*.

MATERIALS AND METHODS

Strains and microbial techniques: The yeast strains used in this work are listed in Table 1. All strains designated "MS" are isogenic with the S288C background. Media for yeast growth as well as general genetic techniques, including mapping, are as described by Rose *et al.* (1990). *Escherichia coli* strains HB101 (Boyer and Roulland-Dussoix 1969) and XL1-Blue (Bullock *et al.* 1987) were used in all bacterial manipulations, and bacterial culture media were as described by Davis *et al.* (1980).

For the semiquantitative plate mating assay, a modification of the standard mating type test protocol was used (ROSE *et al.* 1990; ROSE and FINK 1987). Fresh lawns of a wild-type strain and fresh patches of strains to be tested were inoculated by replica-plating the day before the mating. For mating, strains were replica-plated together onto YPD plates and allowed to mate for 3–5 hr at 23° or 30°. Mating plates were then replica printed to synthetic medium to select for diploid cells. The frequency of diploid formation was compared to *KAR1* and *kar1-1* controls on the same plate. For microscopic assays, approximately 5×10^6 exponentially growing cells of each parent were mixed together and concentrated on a 0.45-μm pore size nitrocellulose filter. The mating mixtures were incubated for 5 hr on YPD at 23° and then fixed in methanol:acetic acid (3:1) for at least 1 hr at 4°. Cells were then washed in phosphate-buffered saline and incubated with the fluorescent DNA-specific dye 4′,6′-diamidino-2-phenylindole (DAPI) to visualize yeast nuclei.

Plasmid constructions and DNA manipulations: Plasmids were constructed by standard methods, and enzymes were used according to the specifications of the manufacturer. The construction of the *kar1-Δ17* allele, which deletes amino acids 190–246, has been previously described (VALLEN *et al.* 1992b). A slightly smaller deletion in this region, *kar1-Δ30*, deletes amino acids 190–236. This allele was constructed by first inserting a *Sac*II linker (Pharmacia, CCGCGG) into the unique *Xmn*I site in *KAR1* (base pair 707) in pMR1295 (VALLEN *et al.* 1992b) to form pM1761. This plasmid was then cut with *Sac*I and *Sac*II, blunted with T4 DNA polymerase, and religated to create *Δ30*.

To analyze the intragenic *kar1-Δ17-SUP* suppressor alleles, genomic DNAs from the *kar1-Δ17-SUP* loci from MS2978 and MS2979 were recovered by gap repair of pMR14 (ROSE and FINK 1987) digested with *Sac*I. Transformation by gapped plasmids is dependent upon templated repair by the chromosomal *kar1-Δ17-SUP* allele. Plasmids were isolated from yeast by the method of HOFFMAN and WINSTON (1987), and the recovery of the *kar1-Δ17-SUP* allele was tested by transforming a *kar1-Δ17* strain, MS2082, with the gap-repaired plasmids. If a plasmid contained a dominant *kar1-Δ17-SUP* allele, it conferred temperature resistance to a *kar1-Δ17* strain upon transformation. The suppressors were mapped to the first 570 bp of the coding sequence, by replacing either the upstream *Hin*dIII-*Sal*I fragment or the downstream *Sal*I-*Eco*RI fragment, with unmutated *kar1-Δ17* sequences. The recombinant plasmids were tested for their ability to suppress the *kar1-Δ17* temperature sensitive phenotype as described above. The *Hin*dIII-*Sal*I fragment containing the mutations was then sequenced from a supercoiled plasmid template by the dideoxy method of SANGER *et al.* (1977) using Sequenase (U.S. Biochemical Corp., Cleveland, Ohio).

The subclones constructed for the analysis of DNA from the multicopy suppressor locus are described below. To sequence the *CDC31* locus from the *DSK1* suppressor strains, genomic DNA from the *CDC31* locus was amplified by polymerase chain reaction (PCR). A single yeast colony was resuspended in PCR buffer (10 mM Tris, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 1 μM upstream and downstream primers) (primers were synthesized by M. FLOCCO, Princeton University). The cell suspension was boiled for 5 min and cooled to room temperature. *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Connecticut) was added (2.5 units) and the reaction was overlaid with 100 μl of light paraffin oil. Amplification (1 min, 94°; 2 min, 42°; 2.5 min, 72°) was performed for 30 cycles. Ten microliters of each reaction were run on a 2% NuSieve GTG low melt agarose (FMC Corporation, Rockland, Maine) gel. The amplified product was excised in a minimal volume, and the gel melted at 65° for 5 min. The amplified DNA in molten agarose (6 μl) was mixed with 3 μl of sequencing primer (10 ng/μl) and boiled for 2 min. Annealing was allowed to occur at 42° for 5 min. DNA was sequenced by the dideoxy method, keeping the samples at 37° to prevent solidification of the agarose.

To recover the *CDC31-16* (*DSK1-6*) mutation on a plasmid, pMR2034 (Figure 7; described below) was digested with *Sal*I and used to transform the *CDC31-16*-containing strain, MS2623. To assay recovery of the suppressor allele, gap-repaired plasmids were transformed into MS2082 and MS2083 and the transformants screened for their ability to grow at 37°. The *CDC31* locus was then sequenced to determine the site of the *CDC31-16* mutation.

Isolation of suppressors: Spontaneous, temperature-resistant (Ts^+) revertants of the *kar1-Δ17* allele were selected in strains MS2082 and MS2083. Independently arising suppressor alleles were isolated by inoculating single colonies into 1-ml YPD cultures and growing them to stationary phase at 23°. These cultures were plated onto prewarmed YPD plates and incubated for approximately 2 days at 37°. To ensure independence of the suppressors, only one Ts^+ colony per plate was pursued. Putative mutants were purified on YPD at 23°, and isolated colonies were retested for the ability to grow at 37°. The suppressor strains were found to have diploidized (see RESULTS). To recover haploid *kar1-Δ17*, Ts^+ strains, we transformed the diploids with *MAT*-containing plasmids of the opposite mating type (B1295 and B1311; gift of K. WEILER and J. BROACH, Princeton University, Princeton New Jersey) to allow the strains to be sporulated.

Isolation and characterization of multicopy suppressor plasmids: Genes that suppress the *kar1-Δ17* allele in multicopy were isolated from a YEp24 genomic library (CARLSON and BOTSTEIN 1982). Transformants of strain MS2087 were selected on SC-URA plates at 23°. Colonies were replica-plated to prewarmed 37° plates and incubated for approximately 36 hr. Putative Ts^+ colonies were picked from the 23° master plate, purified and retested. Plasmids were recovered from Ts^+ strains by the method of HOFFMAN and WINSTON (1987) and plasmid linkage of the Ts^+ phenotype was tested after retransformation into MS2087.

A *Pvu*II fragment (Figure 7) from one of the multicopy suppressor plasmids, pMR1919, was subcloned into pRS406, pRS416 and pRS426 (SIKORSKI and HIETER 1989) digested with *Pvu*II to form the *URA3*-based, integrating, CEN, and 2-μm plasmids, pMR2000, pMR2012 and pMR2032, respectively.

Directed integration of pMR2000 to the "multicopy suppressor locus" was achieved by digestion with *Bam*HI, which cuts the plasmid once, within the insert, before transformation. To analyze the ability of this locus to suppress the temperature sensitivity of *DSK1-6* strains, MS2623 was transformed with pMR2000. Linkage of the "multicopy suppressor locus" to *DSK1* and *HIS3* was demonstrated by integrating pMR2000 into MS52 to form MS2356 and crossing MS2356 to MS2468, MS2474, MS142, and MS1554 (Table 4).

We used a variety of plasmids to determine if *CDC31* or a tightly linked locus was responsible for complementing the temperature sensitivity of *DSK1-6*. Pertinent to this, there is a 216 amino acid open reading frame (ORF) just downstream of *CDC31*. Plasmid JA1 (B. BYERS, University of Washington) contains approximately 1.6 kb of DNA from the *CDC31* locus and includes the downstream ORF (Figure 7). JA1 was constructed by cloning the genomic *Hin*dIII-*Hin*dIII fragment containing *CDC31* into the *Hin*dIII site pRS316 (SIKORSKI and HIETER 1989). To determine which ORF had the ability to suppress the temperature sensitivity of *DSK1-6* containing strains, we constructed deletions and mutations in pMR2012 and JA1 (Figure 7). Two deletions encompassing part or all of the

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                190                 210                 230                 250
KAR1        ··PIINNKSSSQRKSSVALRKQLGKPLPLPYLNSPNSDSTPTLQRKEEVFTDEVLQKKRELIESKWHR...
karlΔ17     ..PIINNK------^----------PVDPK-------------------------------KWHR...
KARlΔ17[1C1] ..PIINNK----------------LVDPK-------------------------------KWHR...
karlΔ30     ..PIINNK--------------------G----------------------LQKKRELIESKWHR...
```

FIGURE 1.—Predicted protein sequence of the *karl-Δ17* mutant and an intragenic suppressor allele. The predicted sequence of wild-type Karlp corresponding to amino acids 185–250 is shown in the top line. The corresponding region of protein encoded by the temperature-sensitive *karl-Δ17* allele is shown in line 2; amino acids 190–246 are deleted and linker sequences span the novel joint. The temperature-resistant intragenic suppressor mutation, *karl-Δ17[1C1]*, causes the change at amino acid 192 shown in line 3. The sequence of the protein encoded by the temperature-sensitive deletion mutation, *karl-Δ30*, encompassing amino acids 190–236, is shown in the bottom line.

CDC31 locus were created by first linearizing pMR2012 by partial digestion with either *Bsm*I or *Pst*I. The linear plasmids were digested with *Hin*dIII and the ends were blunted with Klenow and T4 DNA polymerase. *Sal*I linkers (M. FLOCCO, Princeton University) were ligated to the blunt ends. The ligation mix was heated to 65°, slowly cooled to room temperature to anneal the linkers, and transformed into *E. coli*. This created plasmid pMR2034, which deletes the *Hin*dIII-*Pst*I fragment containing the entire coding sequence of *CDC31*, and pMR2035, which deletes the *Hin*dIII-*Bsm*I fragment including approximately half of the *CDC31* coding sequence (Figure 7). We also isolated a *Hin*dIII-*Ssp*I fragment containing the entire downstream ORF, but only the carboxy-terminal portion of *CDC31* from JA1 and ligated it into pRS416 digested with *Sma*I-*Hin*dIII to form pMR2043 (Figure 7).

In addition to these alleles, two mutations predicted to inactivate the gene downstream from *CDC31* were constructed in JA1. First, a *Sal*I linker, predicted to shift the reading frame within the ORF, was inserted into the unique *Bal*I site of JA1, forming pMR2041. Second, a deletion between *Bal*I and *Sna*BI, within the ORF, was also isolated (pMR2044).

Immunofluorescent and electron microscopy: Immunofluorescent staining of yeast cells was performed by a modification of the methods of ADAMS and PRINGLE (1984) and KILMARTIN and ADAMS (1984), as described by ROSE and FINK (1987). Rabbit antiserum (RAP124) directed against yeast β-tubulin was a generous gift from F. SOLOMON. FITC-conjugated secondary antibodies were purchased from Boehringer Mannheim (Indianapolis, Indiana). The fluorescent DNA-specific dye DAPI was used to visualize yeast nuclei.

For electron microscopy, strains MS2083 (*karl-Δ17*) and MS2623 (*CDC31-16*) were grown to early log phase in YM-1 media (HARTWELL 1967) at 23° and then shifted to 37° for 4 hr. Cells were harvested and processed for electron microscopy as described by BYERS and GOETSCH (1991). Serial sections through the entire nucleus for 15–17 large budded cells were examined for each of the mutant strains.

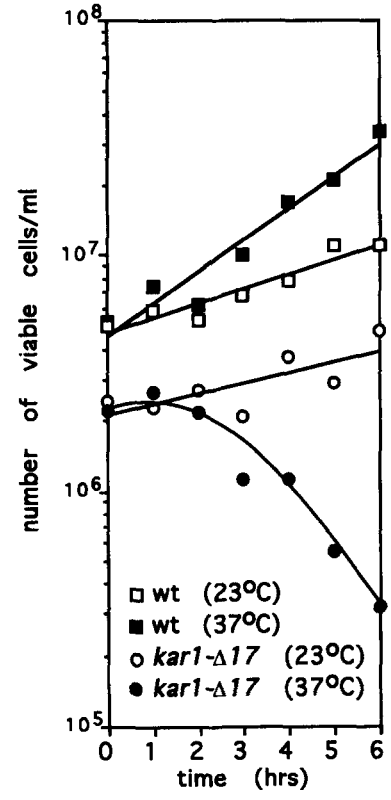

FIGURE 2.—Viability of the *karl-Δ17* mutant at the nonpermissive temperature. A *karl-Δ17* strain (MS2083) and a wild-type *KAR1* strain (MS10) were grown to exponential phase in YPD at 23°. At time zero the cultures were split; half were grown at 23° and half were grown at 37°. Samples were removed every hour and plated on YPD at 23° to determine viability.

RESULTS

Characterization of *karl[ts]* mutants: Isolation of the recessive, temperature sensitive *karl-Δ17* allele was described previously (VALLEN *et al.* 1992b). The *karl-Δ17* allele has amino acids 190–246 deleted and replaced with 5 amino acids due to linker sequences present at the junction (Figure 1). After shift to the nonpermissive temperature (37°), the viability of the *karl-Δ17* strain, MS2083, decreased within 2 hr (Figure 2) reaching approximately 10% within 6 hr. Microscopic examination revealed that the cells arrested with a single large bud and immunofluorescent staining showed only a single vertex of microtubules (data not shown), consistent with a block in SPB duplication (ROSE and FINK 1987). Unlike the previously characterized *karl* alleles, the *karl-Δ17* arrest occurred in the first cell cycle after the temperature shift. Electron microscopic analysis of serial sections through mutant nuclei confirmed the presence of a single monopolar spindle in 13 of 15 large budded cells. The SPB was unduplicated and enlarged (*cf.* Figure 6), similar to the phenotype caused by the previously described *karl* alleles. The remaining two cells had duplicated and separated their SPBs to form short but morphologically normal mitotic spindles.

To define further the functional sequence requirements in this region, we created a smaller deletion, *karl-Δ30*. This allele deletes amino acids 190–236, and adds

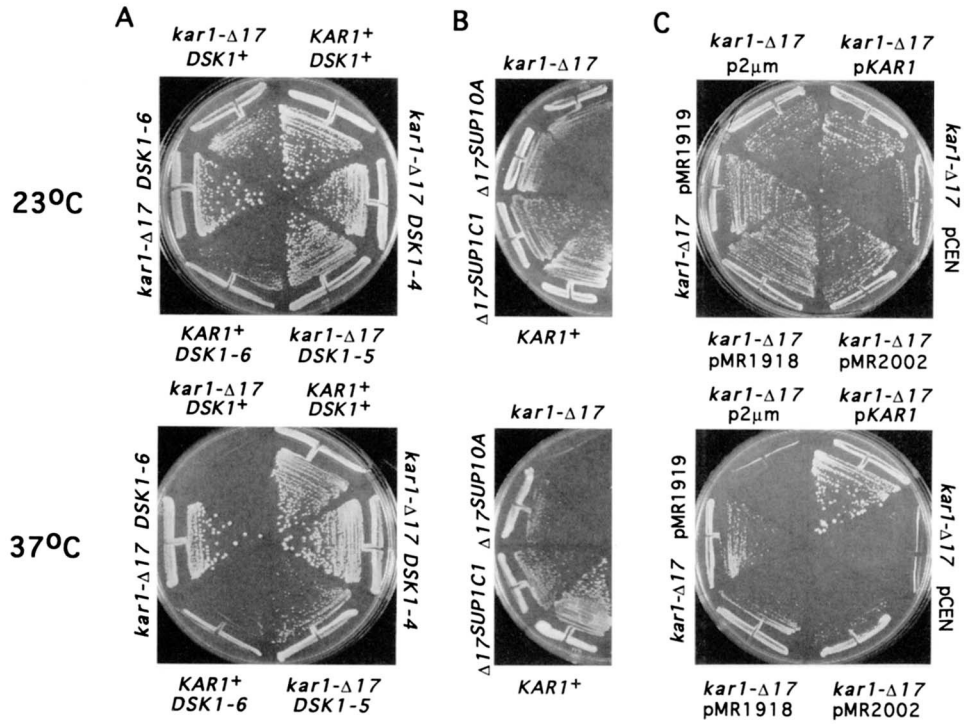

FIGURE 3.—Growth phenotypes of *kar1-Δ17* and temperature-resistant suppressors. (A) Representative strains bearing extragenic suppressors of *kar1-Δ17* are shown. Top, YPD at 23°; bottom, YPD at 37°. With the exception of the *KAR1 DSK1-6* strain, strains bearing suppressor mutations are original isolates and therefore are diploid and heterozygous for the suppressor mutation. Equivalent haploid strains are: (reading clockwise from upper left sector) *kar1-Δ17 DSK1*, strain MS2082; *KAR1 DSK1*, wild-type strain MS52; *kar1-Δ17 DSK1-4*; strain MS3143, *kar1-Δ17 DSK1-5*, strain MS3135; *KAR1 DSK1-6*, strain MS2623, *kar1-Δ17 DSK1-6*, strain MS3137. (B) Growth of the two strains carrying intragenic suppressors on YPD at 23° (top) and 37° (bottom) is shown. Strains: *kar1-Δ17*, strain MS2082; *kar1-Δ17*^{SUP10A}, strain MS2978; *kar1-Δ17*^{SUP1C1}, strain MS2979; *KAR1*, wild-type strain MS52. (C) Multicopy plasmid suppressors of *kar1-Δ17* were isolated from a YEp24-based genomic library (CARLSON and BOTSTEIN 1982). Plasmids were isolated and retransformed into *kar1-Δ17* strain MS2083. Strains were grown on SC-URA to maintain selection for the plasmids. Top, 23°; bottom, 37°. Plasmids were as follows: (reading clockwise from upper right sector) p*KAR1*, pMR76 (wild-type *KAR1* on YCp50); *kar1-Δ17* pCEN, pMR1868 (*CEN* plasmid vector, pRS416), *kar1-Δ17* pMR2002, *kar1-Δ17* pMR1918 and *kar1-Δ17* pMR1919 are different high copy number plasmids containing the wild type *DSK1* gene; *kar1-Δ17* p2 μm, pMR1872 (2 μm vector control pRS426). See Figure 7 for restriction maps of plasmids pMR1918, pMR1919 and pMR2002.

one novel amino acid at the junction as shown in Figure 1. This allele also confers temperature sensitive growth to strains carrying it, however, the phenotype is not as severe as that caused by *kar1-Δ17*.

Isolation and characterization of suppressors of *kar1-Δ17*: To isolate spontaneous suppressors of *kar1-Δ17*, two temperature-sensitive mutant strains, MS2082 and MS2083, were plated at 37°. Temperature-resistant colonies arose at a frequency of $\sim 10^{-8}$. Sixteen *DSK* (*Dominant Suppressor of kar1*) mutants able to grow at 37° were further characterized. In most cases, when the candidate suppressor mutants were crossed to wild-type strains MS37 and MS53, spore viability was less than 30%. The segregation of the *MAT* locus and other markers suggested that the strains carrying *kar1-Δ17* had diploidized (data not shown), consistent with the phenotypes of previously described *kar1* mutations (ROSE and FINK 1987). Therefore, we transformed the diploid revertants with plasmids containing the *MAT* locus of the opposite mating type. Transformation increased spore viability to greater than 75%, consistent with diploidization. For 12 of the putative suppressors, two spore colonies were temperature sensitive and two were temperature resistant, as would be expected for the segregation of a single heterozygous suppressor allele. Therefore, the suppressor mutations arose after diploidization. One revertant, *DSK-9*, yielded only tetrads with four temperature resistant spores. When the *DSK-9* temperature resistant spores were crossed to a *kar1-Δ17* strain, temperature resistance segregated 2:2. Most likely, the *DSK-9* suppressor allele had undergone mitotic recombination in this strain to make it homozygous. For the remaining three putative suppressor strains, no temperature resistant spores were recovered and these were not analyzed further.

Haploid spores containing the *DSK* mutations were recovered and used in all further manipulations. In addition to suppressing the temperature sensitivity (Figure 3), all 13 *DSK* mutations also suppressed the diploidization phenotype of *kar1-Δ17*. All of the *DSK*, *kar1-Δ17* strains could be propagated stably as haploids and gave high spore viability in further crosses (greater than 80%). As expected from their isolation in diploid strains, all of the alleles were demonstrated

TABLE 2

Tetrad analysis of segregation of *kar1-Δ17* and *DSK1*^{sup} alleles in crosses to *KAR1, DSK1* strains

A. Observed phenotypes and frequencies

Cross	No. of tetrads Ts$^+$:Ts$^-$ 4:0	3:1	2:2	1:3	0:4
kar1-Δ17, DSK1 × *KAR1, DSK1*[a]	0	0	28	0	0
kar1-Δ17, DSK1-9 × *KAR1, DSK1*[b]	8	17	3	0	0
kar1-Δ17, DSK1-6 × *KAR1, DSK1*[c]	7	0	20	0	5
KAR1, DSK1-6 × *KAR1, DSK1*[d]	0	0	18	0	0
KAR1, DSK1-9 × *KAR1, DSK1*	20	0	0	0	0

B. Genotypes, and predicted and observed phenotypes for spore progeny from crosses between *kar1-Δ17, SUP1-1* and *KAR1, sup1*$^+$ strains

Tetrad type	Predicted frequency	Genotype of spores	Growth at 37° Expected phenotype[e]	Observed phenotype[f] *DSK1-6*
PD	1	2 *KAR1, sup1*$^+$	+	+
		2 *kar1-Δ17, SUP1-1*	+	+
TT	4	1 *KAR1, sup1*$^+$	+	+
		1 *KAR1, SUP1-1*	+	−
		1 *kar1-Δ17, SUP1-1*	+	+
		1 *kar1-Δ17, sup1*$^+$	−	−
NPD	1	2 *KAR1, SUP1-1*	+	−
		2 *kar1-Δ17, sup1*$^+$	−	−

[a] A *kar1-Δ17*-containing strain suitable for genetic crosses was constructed by integrating the *kar1-Δ17* allele into a diploid, transforming the diploid with a *KAR1* plasmid, and dissecting tetrads to recover a haploid strain containing *kar1-Δ17* on the chromosome covered by a wild-type *KAR1* allele.

[b] The suppression of the *kar1-Δ17* allele by an extragenic suppressor is demonstrated by the recovery of tetratype tetrads, the most frequent class of spores, containing three Ts$^+$ spores. See part B for predicted genotypes and phenotypes. 4:0, parental ditype (PD); 3:1, tetratype (TT); 2:2, nonparental ditype (NPD).

[c] The lack of 3Ts$^+$:1Ts$^-$ tetrads and the recovery of OTs$^+$:4Ts$^-$ tetrads demonstrates that two Ts$^-$ mutations that cosuppress are segregating in the cross. See part B for predicted genotypes and phenotypes. 4:0, PD; 2:2, TT; 0:4, NPD.

[d] The segregation of a single Ts$^-$ mutation in this cross suggests that the *DSK1-6* allele is Ts$^-$ in a *KAR1* strain. Backcrossing Ts$^-$ spores to *kar1-Δ17* containing strains demonstrated unambiguously that the Ts$^-$ phenotype was linked to the *DSK1* suppressor locus.

[e] The expected phenotype is based on the predicted phenotypes for *kar1-Δ17* and a suppressor segregating independently, when the suppressor confers no phenotype in a wild-type *KAR1* strain. This segregation pattern was observed for eight of the nine *DSK1* alleles and both *DSK2* alleles. An example is shown in part A, line 2 for *kar1-Δ17, DSK1-9* × *KAR1, DSK1*.

[f] The observed phenotypes are found for *kar1-Δ17, DSK1-6* × *KAR1, DSK1* as shown in part A, line 3. These phenotypes suggested that *DSK1-6* in a wild-type *KAR1* strain is Ts$^-$. This was confirmed by complementation analysis and backcrosses (see RESULTS).

to be dominant suppressors when crossed back to a *kar1-Δ17* strain.

We determined the number of *DSK* genes by crosses among them. The 13 mutations comprised three linkage groups. The first group, containing two alleles, was completely linked to the *KAR1* locus (Figure 3B). Two groups were unlinked to *KAR1*; the *DSK1* linkage group

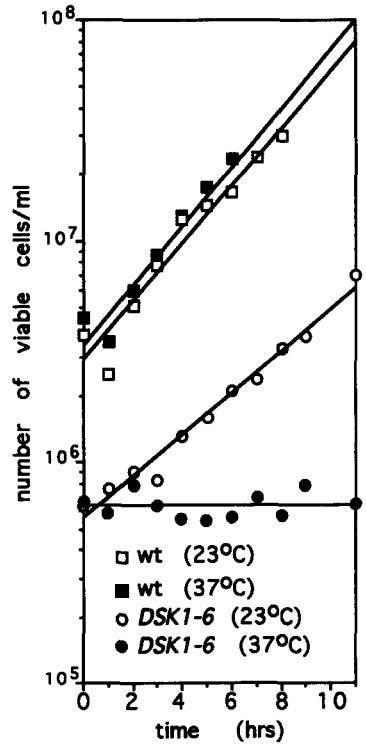

FIGURE 4.—Viability of the *DSK1-6* mutant at nonpermissive temperature. A *DSK1-6* strain (MS2623) and a wild-type *KAR1* strain (MS10) were grown to exponential phase in YPD at 23°. At time zero the cultures were split; half were grown at 23° and half were grown at 37°. Samples were removed every hour and plated on YPD at 23° to determine viability.

contained nine alleles and the *DSK2* linkage group contained two alleles.

Analysis of *kar1-Δ17* pseudorevertants: Suppressor alleles genetically linked to *KAR1* were recovered from genomic DNA by the plasmid rescue technique (ORR-WEAVER *et al.* 1983). The suppressor alleles are designated *kar1-Δ17-SUP* with capital letters signifying the dominance of the temperature resistant phenotype. To confirm that the suppressor mutations were due to mutations in *kar1-Δ17*, centromere-based plasmids containing the *kar1-Δ17* loci from SUP1C1 and SUP10A were introduced into a *kar1-Δ17* strain. The two suppressor loci conferred temperature resistance whereas the original *kar1-Δ17* allele on the same vector did not.

We next determined the location of SUP1C1 and SUP10A within the *KAR1* coding sequence. Replacing restriction fragments of the original *kar1-Δ17* gene with equivalent fragments from the suppressor alleles mapped the mutations to the first 570 bp of *KAR1*. DNA sequence analysis of this region demonstrated the presence of a single mutation in both pseudorevertants. The stronger suppressor, SUP1C1 changed a codon at the fusion joint of *kar1-Δ17*, P192L (Figure 1). SUP10A, a weaker suppressor mutation, was upstream of the novel junction and changed F111Y.

Co-suppression of *kar1-Δ17* and *DSK1-6*: Suppressor mutations often cause secondary phenotypes that

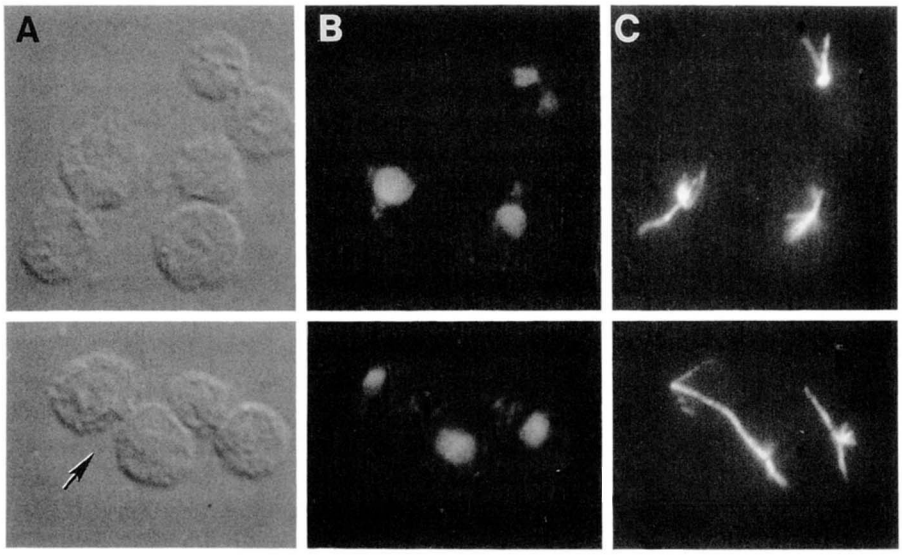

FIGURE 5.—Cell cycle arrest morphology of the *DSK1-6* mutant at the nonpermissive temperature. A *DSK1-6* strain (MS2623) was incubated at 37° for 4 hr. Cells were examined by Nomarski optics (A), DAPI staining of DNA (B) and indirect immunofluorescent staining of microtubules (C). Four cells arrested in the cell cycle are shown. Each was arrested with a large bud, single nucleus and a single vertex of microtubules, consistent with a defect in SPB duplication or separation. For comparison, a fifth large budded cell of wild type appearance is also shown (bottom left, arrow). This cell contained an elongated bipolar spindle and had completed nuclear division.

TABLE 3

Cell cycle arrest of the *DSK1-6* mutant

Genotype	Temp (°C)	Time (hr)	Unbudded mononucleate	Small bud mononucleate	Large bud mitotic	Large bud mononucleate	Unbudded binucleate	Unbudded anucleate
DSK1	23	0	45[a]	31	23	<1	<1	<1
DSK1	37	4	43	41	14	<1	2	<1
DSK1-6	23	0	19	32	9	29	5	6
DSK1-6	37	1	32	12	1	25	4	25
DSK1-6	37	2	22	17	1	27	2	31
DSK1-6	37	3	15	14	2	47	1	20
DSK1-6	37	4	14	11	1	59	1	16
DSK1-6	37	5	19	13	1	47	<1	19
DSK1-6	37	6	14	9	1	53	3	20

DSK1 strain MS10 and *DSK1-6* strain MS2623 were incubated at 23° and 37°. Samples were removed at indicated times and examined by immunofluorescent microscopy to determine the cell and nuclear morphology. Cells with buds smaller than approximately 2/3 the size of the mother cell were classified as small budded cells. Cells containing buds equal to or greater than 2/3 the mother cell were classified as large budded cells. Mitotic cells contained either an elongated nucleus or two nuclei. The novel class of large budded mononucleate cells, contained cells whose buds were usually equal in size to the mother cell.

[a] Numbers in each column correspond to the percentage of cells observed with the indicated morphology. Greater than 200 cells were counted for each time point.

can only be observed in an otherwise wild-type strain (BOTSTEIN and MAURER 1982; ADAMS and BOTSTEIN 1989). Since none of the *kar1-Δ17, DSK* double mutant strains showed any obvious secondary phenotypes, we crossed all of the suppressor alleles into a wild-type *KAR1* strain. In one case, *DSK1-6*, the suppressor mutation proved to cause a secondary phenotype as demonstrated by an unusual pattern of temperature sensitivity segregating in the cross. The unusual segregation pattern (2/3 of the tetrads contained 2 Ts^+ and 2 Ts^- spores, 1/3 contained all 4 spores either Ts^+ or Ts^-) was most easily explained by the presence of two unlinked Ts^- mutations that suppressed each others temperature sensitivity (Table 2). These data suggested that *DSK1-6* was temperature-sensitive in a *KAR1* strain (Figure 3A). To confirm this hypothesis, we backcrossed all four spores recovered from two tetrads predicted to be tetratype asci (2 Ts^+:2 Ts^- spores). When the Ts^- spores were backcrossed to a wild-type strain, temperature sensitivity segregated 2:2 as expected for a single temperature-sensitive locus. Complementation tests demonstrated that one of the Ts^- mutations was *kar1-Δ17*, while the other was not, confirming the hypothesis that two different temperature-sensitive mutations were present. The Ts^+ spores from the tetratype tetrads behaved differently from one another when crossed to wild type. One Ts^+ spore segregated only Ts^+ progeny, whereas the other Ts^+ spore yielded both Ts^+ and Ts^- spores, in a pattern identical to that seen for the original cross. This confirmed that one of the Ts^+ spores contained two Ts^- mutations (*kar1-Δ17* and *DSK1-6*) that were reciprocally suppressed. Further analysis demonstrated that the temperature sensitivity of *DSK1-6* was recessive to wild-type *DSK1*, in contrast to the dominance of its suppressor phenotype.

To elucidate the function of *DSK1* during normal growth, we characterized the phenotype caused by the *DSK1-6* allele. The growth of a *DSK1-6, KAR1* strain

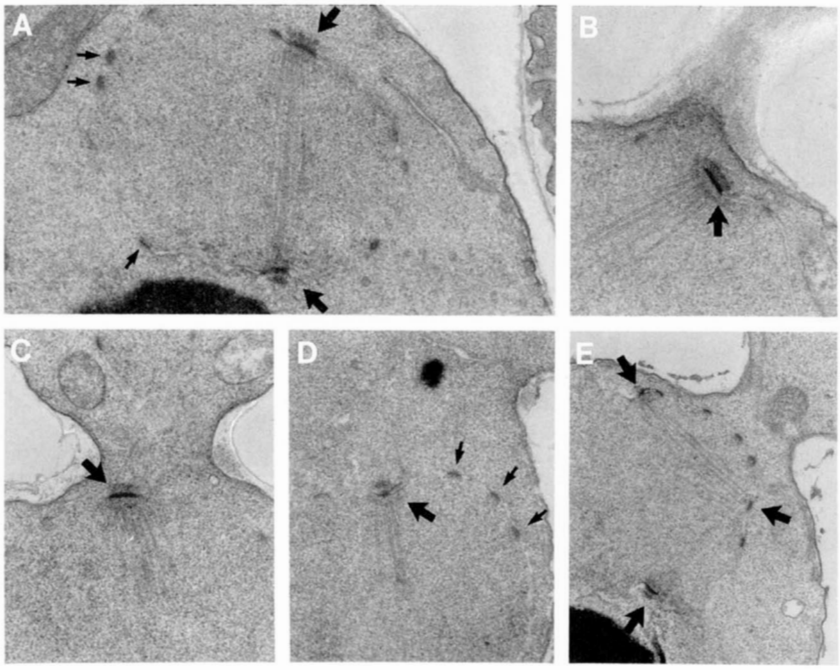

FIGURE 6.—Electron microscopic analysis of the *kar1-Δ17* and *DSK1-6* mutants. A *kar1-Δ17* mutant strain, MS2083, and a *DSK1-6* mutant strain, MS2623, were grown at 23°, and then shifted to 37° for 4 hr prior to processing for electron microscopy. Complete serial sections through the nuclei of 15–17 large budded cells were examined for each mutant. In panel A, a normal short bipolar spindle is seen in the *DSK1-6* mutant. In panel B, a representative unduplicated, enlarged SPB in the *kar1-Δ17* mutant is shown. Panels C and D show representative unduplicated SPBs for the *DSK1-6* mutant. In many of the *DSK1-6* mutant cells, the unduplicated SPBs had enlarged half-bridges associated with them (D), while others did not (C). Panel E shows a tripolar spindle seen in a *DSK1-6* mutant. Large arrows, SPBs. Small arrows, nuclear pores.

(MS2623) arrested quickly after shift to 37° (Figure 4). Microscopic examination demonstrated that cells accumulated either with large buds or as unbudded cells. DAPI staining of the DNA and indirect immunofluorescent staining of tubulin (Figure 5) established that greater than 50% of the cells arrested with a large bud, a single nucleus, and a single vertex of microtubules (Table 3). An additional 20% of the cells accumulated as aploid cells, devoid of nuclear DNA. Some of the cells scored as being normal and unbudded had DAPI staining that was smaller than normal, or fragmented, suggesting that cytokinesis may have occurred without wild-type nuclear division. It is therefore likely that 70% is an underestimate of the frequency of arrested and abnormal cells. These phenotypes are reminiscent of loss of *KAR1* function (ROSE and FINK 1987; also see above) and are consistent with a cell cycle block at SPB duplication or separation.

Electron microscopy was used to determine the SPB morphology of the arrested cells (Figure 6). Complete serial sections through the nuclei of 17 cells demonstrated that the majority of the cells (12) arrested with monopolar spindles. The single, unduplicated SPBs were often larger than those seen in wild-type cells and frequently were associated with a half-bridge. Of the remaining five cells analyzed, four had bipolar spindles and one had a tripolar spindle. The arrest phenotype caused by *DSK1-6* strongly suggested that the wild-type gene product is required for the same stage in the cell cycle as *KAR1*, namely SPB duplication.

Identification of multicopy suppressors of *kar1-Δ17*: To isolate genes that could suppress *kar1-Δ17* when present in multicopy, strain MS2087 was transformed with a yeast genomic library on a 2 μm-based vector (CARLSON and BOTSTEIN 1982) and transformants able to grow at 37° were isolated (Figure 3C). Seven plasmids, which conferred temperature-resistant growth upon retransformation into MS2087, were recovered from a screen of 15,000 transformants. When these were characterized by restriction enzyme analysis, three were found to contain the *KAR1* locus. Although overexpression of *KAR1* is toxic (ROSE and FINK 1987), some strains are able to tolerate *KAR1* on 2 μm-based vectors. The remaining four plasmids were comprised of three different, overlapping fragments of yeast DNA. Together, the multicopy suppressor plasmids (pMR1918, pMR1919, pMR1920 and pMR2002) contained inserts that defined a region of overlap of approximately 3.0 kb (Figure 7A), which will temporarily be called *MSL* (*M*ulticopy *S*uppressor *L*ocus).

Multicopy suppressors contain the *DSK1* locus: A variety of criteria established that *MSL* was allelic to *DSK1*. First, all of the multicopy *MSL* plasmids suppressed the temperature sensitivity of the *DSK1-6* allele in a *KAR1* background. Second, to ensure that *MSL* in single copy could suppress *DSK1-6*, we subcloned a genomic *Pvu*II fragment from pMR1919 onto a YIp vector. The YIp-*MSL* plasmid was transformed into the *DSK1-6* mutant. Integration to the *MSL* locus was directed by restriction enzyme cleavage in the insert DNA. Of nine independent transformants of the *DSK1-6*, eight were temperature-resistant. Therefore, a single extra copy of the *MSL* locus can suppress the defect associated with *DSK1-6*. Based on the results described below, it is likely that the single Ura^+, Ts^- transformant is the result of gene conversion of either *DSK1* to *DSK1-6* or *ura3-52* to *URA3*.

Third, *DSK1* and *MSL* were found to be genetically linked. To establish linkage, we integrated the *URA3*

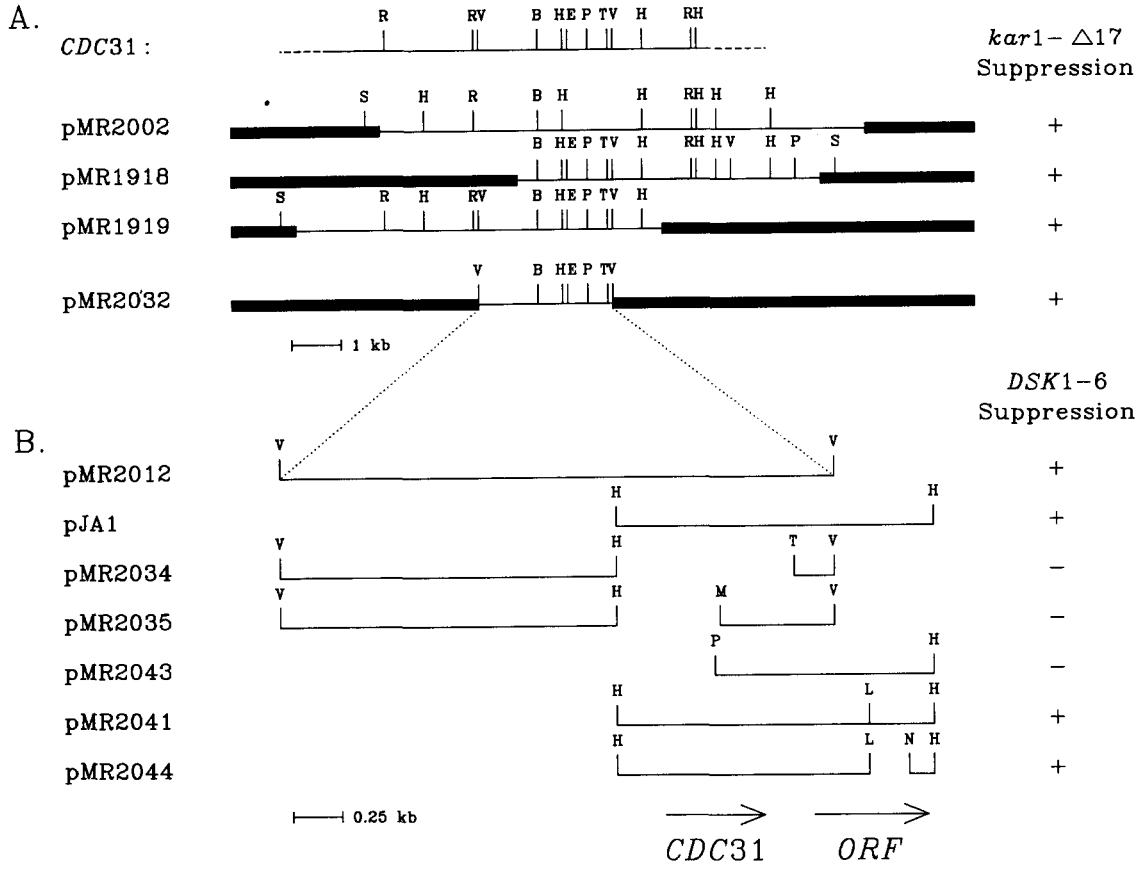

FIGURE 7.—Identification of the *MSL* suppressor gene as *CDC31*. (A) Localization of the region required for multicopy suppression of *kar1-Δ17*. Top line depicts the restriction map of the *CDC31* locus from BAUM *et al.* (1986). Three plasmids (pMR2002, pMR1918 and pMR1919) isolated as multicopy suppressors of *kar1-Δ17* from the YEp24 genomic plasmid library (CARLSON and BOTSTEIN 1982) are shown. A fourth plasmid, pMR1920, was also recovered; it was identical to pMR1918. The *Pvu*II-*Pvu*II fragment from pMR1919 was subcloned into the 2 μm-based plasmid, pRS426 (SIKORSKI and HIETER 1989), to form pMR2032. All four 2 μm-based plasmids suppressed *kar1-Δ17* as indicated. Thick lines indicate vector sequences; thin lines indicate yeast genomic DNA. (B) *CDC31* on *CEN*-based plasmids suppresses *DSK1-6*. Plasmids were tested by transformation into the temperature sensitive *DSK1-6* strain, MS2623. pMR2012 was constructed by subcloning the *Pvu*II-*Pvu*II fragment from pMR1919 into the *CEN*-based plasmid pRS416. Plasmid JA1 contains the *Hin*dIII-*Hin*dIII fragment encompassing the *CDC31* locus in vector pRS316. The remaining five plasmids were produced from pMR2012 or JA1 either by subcloning fragments or constructing deletion derivatives. The ability of the constructs to suppress the temperature sensitivity of *DSK1-6* is shown on the right, and the location of the *CDC31* gene and the downstream *ORF* (open reading frame) is shown at the bottom of the figure. The suppression pattern of the constructs identifies the region required for suppression of *DSK1-6* as *CDC31*. Restriction enzyme sites are designated as follows: B, *Bam*HI; E, *Spe*I; H, *Hin*dIII; L, *Bal*I; M, *Bsm*I; N, *Sna*BI; P, *Ssp*I; R, *Eco*RI; S, *Sal*I; T, *Pst*I; V, *Pvu*II.

containing YIp-*MSL* plasmid at the *MSL* locus in a wild-type strain, giving MS2356. Stable Ura$^+$ transformants were crossed to the *DSK1-6* strains MS2468 and MS2474 and the progeny from these crosses were examined (Table 4). Both Ts$^-$ and Ura$^+$ segregated 2:2. No Ura$^+$, Ts$^-$ recombinant spores were observed in 23 tetrads, demonstrating tight linkage (<2 cM) between *MSL* and *DSK1*. From these data, we conclude that the *DSK1* linkage group identifies the same locus as the multicopy plasmid suppressors.

Identification of *DSK1* as *CDC31*: Restriction enzyme analysis of the *DSK1* locus (Figure 7), as well as genetic linkage to *HIS3* (Table 4), suggested that *DSK1* was likely to be *CDC31*. We used three approaches to prove that *DSK1* was allelic to *CDC31*. This was particu-

TABLE 4

Genetic mapping of the *DSK1* locus

Cross	PD	NPD	TT	Linkage[a]
DSK1 × "*MSL*"[b]	23	0	0	<2 cM
HIS3 × "*MSL*"[c]	12	0	10	23 cM
URA3 × "*MSL*"[d]	3	1	7	Unlinked
ADE2 × "*MSL*"[e]	1	5	17	Unlinked

PD is parental ditype, NPD is nonparental ditype, TT is tetratype.
[a] Determined by the formula of PERKINS (1949).
[b] Analyzed by assaying the Ts$^-$ phenotype of *DSK1-6* and Ura$^+$ phenotype due to the integration of *URA3* at the *MSL* locus. Data are summed from the crosses of MS2468 and MS2474 to MS2356.
[c] Data from MS2356 and MS1554.
[d] Data from MS2356 and MS142.
[e] Data from MS2356 and MS1554.

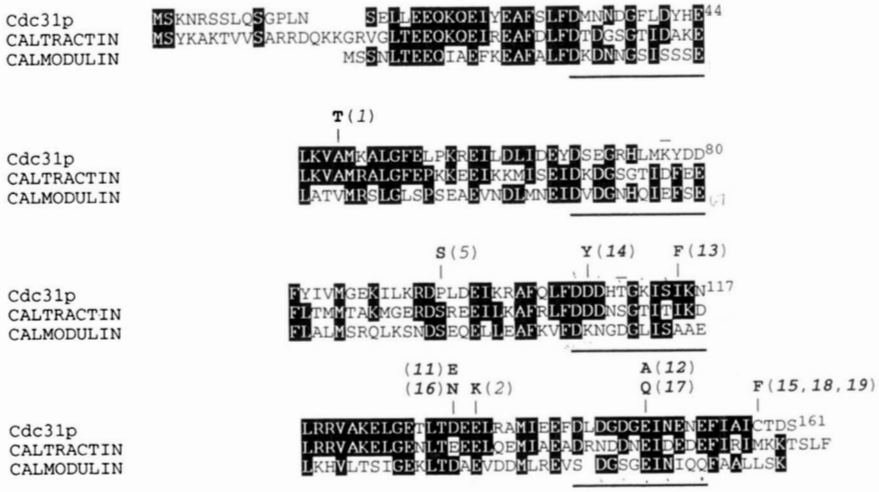

FIGURE 8.—DNA sequence of mutant *CDC31* alleles. Comparison of the predicted amino acid sequences of yeast Cdc31p, Chlamydomonas caltractin/centrin, and yeast calmodulin. Amino acid identities between any two or three of the proteins are shown as white characters on black. Cdc31 is 51% identical to caltractin/centrin and 37% identical to calmodulin. The lineup was created with the Pileup program (Genetics Computer, Inc.). The sequences corresponding to the putative Ca^{2+} binding pockets in calmodulin are underlined. In Cdc31p, only the first and fourth binding pockets are predicted to bind Ca^{2+} (BAUM *et al.* 1986). The numbers at the beginning and end of each line refer to the amino acid residue number in Cdc31p. Two differences between our sequence and that of BAUM *et al.* are demarcated by overlines at amino acids 77 and 110; the amino acids shown are the ones predicted from our sequence analysis. Mutant *CDC31* alleles we and others have isolated are shown as changes above the *CDC31* sequence. The numbers in parentheses correspond to the allele numbers. Alleles *1*, *2* and *5* (light type) are previously isolated recessive temperature sensitive alleles that we have sequenced in this work. Alleles *11* through *19* were isolated in this work as dominant suppressors of *kar1-Δ17*. The alleles *CDC31-12*, *14*, *16* and *17* suppress a complete deletion of the *KAR1* locus.

larly important because another open reading frame was located <200 bp downstream of the *CDC31* gene. First, we analyzed the ability of subclones to suppress the temperature sensitivity of *kar1-Δ17* and *DSK1-6* (Figure 7B). Both mutations were suppressed by subclones containing intact *CDC31* but not the downstream open reading frame. Second, we recovered the *CDC31* locus from a *DSK1-6* strain by gap repair (ORR-WEAVER *et al.* 1983) onto a CEN-based plasmid, pMR2034 (Figure 7B). Transformants of the Ts^- *kar1-Δ17* strain, MS2083, with the resulting plasmid, pMR2223, were Ts^+. Transformants of the same strain with wild-type *CDC31* remained Ts^-. Finally, we analyzed the DNA sequences of the *CDC31* loci from the *DSK1* strains and compared them to the sequence of the *CDC31* locus in the original *kar1-Δ17* strains, MS2082 and MS2083, as well as to a wild-type strain, MS10. In each of the nine *DSK1* mutants, a single mutation was found in the *CDC31* locus (Figure 8). All of the suppressor mutations alter amino acids in the carboxy-terminal domain of Cdc31p. Together, these data demonstrate that the *DSK1* suppressor mutations are alleles of the *CDC31* gene. We therefore renamed the *DSK1* suppressor alleles *CDC31-11* through *CDC31-19*, using capital letters to demonstrate their isolation as dominant suppressors.

Although the suppressors were independently isolated, three (*CDC31-15*, *CDC31-18* and *CDC31-19*) resulted in the same amino acid alteration, C158F. In two cases, we isolated two different alleles that altered the same amino acid. *CDC31-16* (*DSK1-6*) and *CDC31-11* changed D131 to N and E, respectively. *CDC31-12* and *CDC31-17* changed E148 to A and Q, respectively. Finally, *CDC31-13* changed I115 to F, and *CDC31-14* changed D107 to Y.

We found two discrepancies between our wild-type *CDC31* sequence and the published sequence (BAUM *et al.* 1986). Since our wild-type strain (S288C) was different from the published strain, we sequenced *CDC31* from different genetic backgrounds (strains PB9-33B, A32-17A and 12-2B-2D from B. BYERS). All three strains had the same sequence as our wild-type strain. The sequence changes are demarcated in Figure 8 with overlines; L77 is changed to K and I110 is changed to T. K77 is found in the second potential Ca^{2+}-binding loop. This loop was not predicted to have high affinity for Ca^{2+} (BAUM *et al.* 1986), and the change in sequence does not alter this prediction. T110 is found in the third potential Ca^{2+} binding loop, which like loop II, was also not predicted to have high affinity for Ca^{2+}. However, based on homology with other Ca^{2+}-binding proteins, the presence of a polar amino acid at residue 110 increases the probability that this loop binds Ca^{2+} (STRYNADKA and JAMES 1989).

We determined the DNA sequence of the previously isolated temperature sensitive *cdc31* mutations. In contrast to the strong clustering of the $CDC31^{SUP}$ alleles, the three $cdc31^{ts}$ mutations mapped to different regions of the protein (Figure 8). The *cdc31-1* allele changes A48T. The *cdc31-5* allele changes P94S. The *cdc31-2* (E133K) maps in close proximity to the

CDC31^{SUP} alleles. Nevertheless, *cdc31-2*, like *cdc31-5* and *cdc31-1*, is not a dominant suppressor of the *kar1-Δ17* mutation.

Surprisingly, the novel amino acid found in *cdc31-5*, S94, corresponds to the wild-type residue found in both caltractin/centrin (HUANG *et al.* 1988b) and calmodulin (DAVIS *et al.* 1986) (see Figure 8). In calmodulin, the serine is part of a four residue region that is thought to form a flexible hinge within the central α-helix that separates the amino and carboxy-terminal Ca^{2+}-binding lobes (see KLEE *et al.* 1980; BABU *et al.* 1988; STRYNADKA and JAMES 1989; IKURA *et al* 1991). The presence of the wild-type proline might be expected to produce a more rigid kink relative to the serine in this region of Cdc31p. The temperature sensitive phenotype caused by *cdc31-5* suggests that the specific comformation of Cdc31p caused by P94 may be essential for wild-type *CDC31* function.

Some of the dominant mutations bypass completely the mitotic requirement for *KAR1*: To assess the degree of allele specificity, we determined the ability of the *CDC31*^{SUP} alleles to suppress other mutations in *KAR1*. A *CDC31-16* strain (MS2623) was crossed to a strain (MS1697) containing a *kar1* null allele covered by wild-type *KAR1* on a plasmid containing the *URA3* gene. The null allele, *kar1-Δ2*, deletes amino acids 15–392 from the 433 amino acid protein (VALLEN *et al.* 1992b). The parental strain, MS1697, is dependent upon the presence of the plasmid containing *KAR1* because *KAR1* is essential, and the strain is therefore 5-fluoroorotic acid-sensitive (5-FOA^S). In crosses between MS1697 and wild-type strains, 5-FOA^S segregates 2:2 in complete tetrads. Surprisingly, in crosses between MS2623 (*CDC31-16*, *KAR1*) and MS1697 (*CDC31*, *kar1-Δ2*), tetrads containing three or four 5-FOA-resistant (5-FOA^R) spores were recovered. Therefore, *CDC31-16* suppressed the lethality associated with *kar1-Δ2*. Both Southern blot analysis and PCR amplification confirmed that some spores contained only the *kar1-Δ2* allele (data not shown). Like *kar1-Δ17*, the *kar1-Δ2* allele also suppressed the temperature sensitive phenotype of *CDC31-16*, since strains carrying *kar1-Δ2* and *CDC31-16* were Ts$^+$.

Like its suppression of *kar1-Δ17*, *CDC31-16* was dominant for its suppression of *kar1-Δ2*. To provide a wild-type *CDC31* allele, a diploid strain, MS1648, heterozygous for *kar1-Δ2*, and homozygous for *CDC31* and *ura3-52*, was transformed with a CEN-based plasmid containing *CDC31-16* and *URA3* (pMR2223). After sporulation of the diploid transformants, tetrads with two, three and four viable spores were recovered. In tetrads with more than two viable spores, PCR amplification and Southern blot analysis confirmed that two spores contained wild-type *KAR1* on the chromosome, and one or two spores contained the *kar1-Δ2* allele. Viable spores with the chromosomal *kar1-Δ2* allele were always Ura$^+$ and dependent upon the presence of the *CDC31-16* plasmid (pMR2223) for viability, as shown by their sensitivity to 5-FOA. When strain MS1648 was transformed with control plasmids pRS316 (vector only) or pJA1 (wild-type *CDC31*) and sporulated, spore viability segregated 2:2. As expected, none of the viable spores contained the *kar1-Δ2* allele. Therefore, *CDC31-16*, but not wild-type *CDC31*, can bypass the mitotic requirement for *KAR1* function, and furthermore, suppression by *CDC31-16* can occur in the presence of wild-type *CDC31*.

We determined whether other *CDC31*^{SUP} alleles could also suppress an almost complete deletion of the *KAR1* locus by crossing *kar1-Δ17*, *CDC31*^{SUP} strains to a *kar1-Δ2* strain (either MS1697 and MS1699) and analyzing the meiotic progeny. A subset of the *CDC31*^{SUP} alleles (*CDC31-12*, *CDC31-14*, *CDC31-16* and *CDC31-17*) suppressed the mitotic requirement for *KAR1* function, as demonstrated by the recovery of viable *CDC31*^{SUP}, *kar1-Δ2* spores. Only the strongest suppressors of the *kar1-Δ17* allele (as judged by the growth of *kar1-Δ17*, *CDC31*^{SUP} strains at 37°, *cf.* Figure 3A) were able to suppress a *kar1* null allele. Weaker suppressors of *kar1-Δ17* (*CDC31-11*, *CDC31-13* and *CDC31-15*) did not suppress *kar1-Δ2*. In addition, both *DSK2* alleles suppressed the mitotic requirement for *KAR1* function, since they could also suppress a *kar1* null allele. In contrast, the multicopy *CDC31* plasmids (pMR1918, pMR1919 and pMR2002) were unable to suppress a complete deletion of the *KAR1* locus. This is consistent with the relatively weak suppression of the *kar1-Δ17* temperature sensitive phenotype by the multicopy plasmids compared to the genomic *CDC31* and *DSK2* mutations.

We also assayed the ability of the *CDC31*^{SUP} alleles to suppress a deletion of the "SPB region," which is slightly larger than the region deleted in *kar1-Δ17*. The *kar1-Δ18* allele deletes amino acids 190–277 (VALLEN *et al.* 1992b). Strains carrying this allele as the only *KAR1* locus normally are inviable. The same subset of *CDC31*^{SUP} alleles that suppressed *kar1-Δ2* was able to suppress the inviability associated with the *kar1-Δ18* mutation.

A subset of the *CDC31*^{SUP} strains are supersensitive to *KAR1* gene dosage: Although their ability to suppress a complete deletion of *KAR1* suggested that some of the *CDC31*^{SUP} alleles were simple "bypass" suppressors, other aspects of their behavior suggested that the suppressor alleles did not make the cell completely independent of *KAR1* function. One example of this was the suppression of the temperature sensitivity of *CDC31-16* by *kar1* deletion alleles. To further investigate this phenomenon, we transformed a *CDC31-16* strain with plasmids expressing increased levels of Kar1p. These plasmids caused *CDC31-16* strains to be extremely slow growing (Figure 9, Table 5). The same plasmids had no effect on wild-type *CDC31* strains. The supersensitivity to *KAR1* gene dosage was correlated with the strength of the suppressor allele. Strains containing the

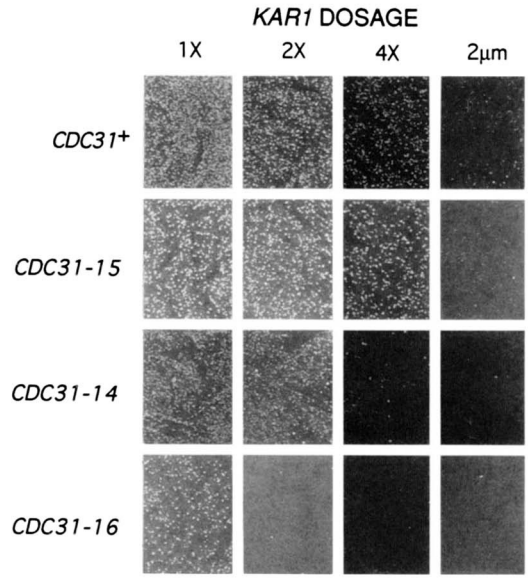

FIGURE 9.—Sensitivity of *CDC31* strains to *KAR1* gene dosage. Representative *CDC31*^{SUP}, *KAR1* strains were transformed with plasmids, plated at 23° on selective media, and photographed after 4 days. Representative regions of the transformation plates are shown. In the first column, 1× *KAR1*, strains were transformed with YCp50, the centromeric vector control. In the second column, 2× *KAR1*, strains were transformed with pMR76, a YCp50-based, *KAR1* plasmid. In the third column, 4× *KAR1*, strains were transformed with pMR711, a YCp50-based plasmid containing the *KAR1-Xba* allele. This mutation results in approximately threefold higher levels of Kar1p translation (VALLEN 1992). In the fourth column, 2 μm *KAR1*, strains transformed with pMR68, a 2 μm-based plasmid containing *KAR1* (ROSE and FINK 1987).

CDC31-14 allele, a weaker suppressor than *CDC31-16* (*cf.* Figure 3, showing weaker suppression of *kar1-Δ17* at 23°), were less sensitive to *KAR1* gene dosage than *CDC31-16*. Strains containing *CDC31-15*, which cannot suppress a *kar1* null, were not supersensitive to *KAR1* gene dosage (Figure 9, Table 5).

Suppression is specific to the mitotic function of *KAR1*: In addition to its role in SPB duplication, *KAR1* is important for nuclear fusion. The region of *KAR1* that mediates SPB duplication is distinct from the region required for karyogamy (VALLEN *et al.* 1992b). We therefore determined whether the *DSK* mutations also suppressed the requirement for *KAR1* in nuclear fusion. Viable *CDC31*^{SUP}, *kar1-Δ2* strains were tested for their ability to form diploids when crossed to a wild-type strain. All *CDC31*^{SUP}, *kar1-Δ2* strains were as deficient for mating as the *kar1-1* and the *kar1-Δ13* karyogamy defective deletion strains. The *DSK2*, *kar1-Δ2* strains were also equally defective in karyogamy as the two *kar1* strains (data not shown). The most straightforward explanation of these results is that *CDC31*^{SUP} and *DSK2* do not suppress the karyogamy defect associated with loss of *KAR1* function. Furthermore, the severity of the defect is consistent with the suggestion that the karyogamy defective *kar1* alleles behave as null alleles for this function (VALLEN *et al.* 1992b) since *kar1-Δ13* and *kar1-1* are as defective as *kar1-Δ2*.

TABLE 5

Growth phenotype of *CDC31* mutants with various *KAR1* gene dosages

	KAR1 gene dosage[a]					
Genotype	*kar1-Δ2*	*kar1-Δ17*	*KAR1*	2× *KAR1*	4× *KAR1*	2 μm *KAR1*
CDC31	−	±	+	+	+	±
CDC31-15	−	+	+	+	+	±
CDC31-14	+	+	+	+	±	−
CDC31-16	+	+	±	−	−	−

[a] Strains were assayed for their ability to grow on SC-URA plates (under plasmid selection) at 23°. *kar1-Δ2* is a complete deletion of the *KAR1* locus; *kar1-Δ17* is the temperature sensitive *KAR1* allele used for the isolation of suppressors. The ability of the *CDC31* alleles to suppress a *kar1*^{null} allele was determined by crossing a strain containing the *CDC31* allele of interest to MS1697 or MS1699 and analyzing the meiotic progeny. Strains containing the various *CDC31* alleles with *kar1-Δ17* were constructed by backcrossing strains containing the *CDC31* allele and *kar1-Δ17*. To construct strains with increasing amounts of *KAR1*, we first backcrossed the original suppressor containing strains (*CDC31*^{SUP}, *kar1-Δ17*) to a wild-type (*CDC31*, *KAR1*) strain and recovered *CDC31*^{SUP}, *KAR1* meiotic progeny. We then transformed *CDC31*^{SUP}, *KAR1* strains with YCp50 (1X *KAR1*); pMR76 (*KAR1* on YCp50; 2X *KAR1*); pMR711 (*KAR1-Xba*, a mutation causing an approximately 3-fold increase in Kar1p levels (VALLEN 1992), on YCp50; 4X *KAR1*); pMR68 (*KAR1* on 2 μm-based plasmid) (ROSE and FINK 1987). (+), growth indistinguishable from wild-type strain; (±), noticeable growth defect, comparable to a wild-type *CDC31* strain transformed with *KAR1* on 2 μm-based plasmid; (−), severe growth defect, more compromised than a wild-type *CDC31* strain transformed with *KAR1* on 2 μm-based plasmid.

In contrast to the results obtained for the *kar1-Δ2* alleles, strains carrying the *CDC31*^{SUP} alleles and *kar1-Δ18*, deleted for residues 191–277, were karyogamy proficient (data not shown). This is consistent with an intragenic complementation test, which demonstrated that the *kar1-Δ18* allele provides functional protein containing the region of *KAR1* required for karyogamy (VALLEN *et al.* 1992b).

We also tested the ability of *CDC31-19* and *CDC31-16* to suppress three karyogamy defective mutations, *kar1-1* and two viable deletions, *kar1-Δ13* and *kar1-Δ15*. Deletion *kar1-Δ13* removed residues 15 through 192 and *kar1-Δ15* removed residues 106–192. Two types of crosses were performed; *CDC31*^{SUP}, *kar1* strains were crossed to wild-type *CDC31*, *KAR1* strains, and, *CDC31*^{SUP}, *KAR1* strains were crossed to *CDC31*, *kar1* strains. *CDC31-19* had no effect on the karyogamy defects associated with *kar1-1*, *kar1-Δ13*, and *kar1-Δ15* in either type of cross.

Surprisingly, two observations indicated that *CDC31-16* interferes with nuclear fusion. First, *CDC31-16* was found to exhibit a mild defect in nuclear fusion when crossed to the wild type. The defect in nuclear fusion was particularly severe in crosses where both parents were *CDC31-16*. Second, *CDC31-16* exacerbated the mating defect of all three karyogamy defective *kar1* alleles in crosses of the type *CDC31-16*,

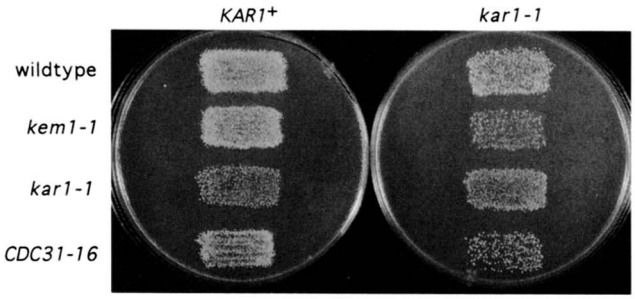

FIGURE 10.—Enhancement of the *kar1* karyogamy defect by *CDC31-16*. Plate matings were performed to demonstrate the enhancement of the *kar1-1* karyogamy defect seen in matings to *CDC31-16*. Strains, from top to bottom: wild-type strain, MY2901; *kem1-1* strain, MY2902; *kar1-1* strain, MS739; *CDC31-16* strain. Patches were mated with wild-type (MY768; left) or *kar1-1* mutant (MS1269; right) lawns on YPD medium for 3 hr prior to replica plating to select for growth of diploids.

KAR1 × *CDC31*, *kar1* (*cf.* Figure 10 and Table 6). For example, in a cross containing *kar1-1*, when *CDC31-16* was also present, the residual nuclear fusion was reduced from 25 to 9%. This defect was as severe as that seen with a previously identified *kar1* enhancing mutation, *kem1-1* (KIM *et al.* 1990). *CDC31-16 kar1-1* strains also had a slightly enhanced mating defect when crossed to a wild-type strain as compared to a *CDC31 kar1-1* strain crossed to wild type. However, when *CDC31-16 kar1-Δ13* or *CDC31-16 kar1-Δ15* strains were crossed to wild type, the strains were no more defective than *kar1-Δ13* or *kar1-Δ15* single mutants (data not shown). Analysis of the *CDC31-16 kar1* double mutants revealed that both *kar1-Δ13* and *kar1-Δ15* suppress the temperature sensitive phenotype of *CDC31-16*, while *kar1-1* does not (Table 6B). Thus the enhancement of the karyogamy defect caused by *CDC31-16* in combination with certain *kar1* alleles was correlated with the suppression of *CDC31-16* by the mutant *KAR1* locus.

Recessive alleles of *cdc31* do not cause defects in mating, indicating that Cdc31p normally plays no essential role in nuclear fusion. However, the *CDC31-16* mutation does cause a defect in nuclear fusion and the effects of *CDC31-16* are sensitive to the presence of specific *kar1* mutations as well as to their configuration in the cross. Taken together, these data raise the possibility of additional genetic interactions between *KAR1* and *CDC31* during mating and suggest that Cdc31-16p may interfere with Kar1p's normal function in nuclear fusion.

TABLE 6

Karyogamy enhancing defect caused by *CDC31-16*

A. Microscopic examination of *CDC31-16* and *kar1-1* zygotes

Genotype		*n*	Percent unfused nuclei
KAR1, CDC31	× *KAR1, CDC31*	125	1
KAR1, CDC31-16	× *KAR1, CDC31*	282	17
kar1-1, CDC31	× *KAR1, CDC31*	102	75
kar1-1, CDC31	× *KAR1, CDC31-16*	193	91
kar1-1, CDC31-16	× *KAR1, CDC31*	312	85
KAR1, CDC31-16	× *KAR1, CDC31-16*	118	57

B. Phenotypes of *CDC31-16, kar1* double mutants

Genotype	Tested by cross to	Ts+ [a]	Kem+ [b]
CDC31-16, KAR1	*kar1-1*	–	–
CDC31-16, KAR1	*kar1-Δ13*	–	–
CDC31-16, KAR1	*kar1-Δ15*	–	–
CDC31-16, kar1-1	*KAR1*	–	–
CDC31-16, kar1-Δ13	*KAR1*	+	+
CDC31-16, kar1-Δ15	*KAR1*	+	+

[a] Phenotype refers to strain carrying alleles listed in Genotype. (–), temperature sensitive growth at 37°C; (+), temperature resistant growth at 37°.

[b] Phenotype refers to strain carrying alleles listed in Genotype. (–), karyogamy enhancing phenotype (*i.e.*, decreased nuclear fusion), (+), no karyogamy enhancing phenotype (*i.e.*, levels of nuclear fusion comparable to that caused by *kar1* mutation alone).

DISCUSSION

KAR1 plays an essential role during mitotic growth and is important for nuclear fusion. Cytological examination of strains carrying temperature sensitive loss of function *KAR1* alleles (ROSE and FINK 1987), indicated that *KAR1* is required for SPB duplication. In addition, Kar1-β-galactosidase hybrid proteins localize to the newly synthesized SPB in mitotically growing cells (VALLEN *et al.* 1992a). The protein domain required for SPB duplication is coincident with the region that is both necessary and sufficient to localize Kar1-β-galactosidase hybrids to the SPB (VALLEN *et al.* 1992b). A small deletion within this region created the temperature sensitive *kar1-Δ17* allele that we have utilized for suppressor analysis.

Several properties of *kar1-Δ17* indicated that it would be amenable to suppressor genetic analysis. First, *kar1-Δ17* strains quickly stopped growing at the nonpermissive temperature, suggesting that the mutant protein is temperature sensitive for stability. Second, since *kar1-Δ17* is a small deletion, true reversion events cannot occur. Finally, *kar1-Δ17* is located in a region critical for *KAR1*'s association with the SPB. Therefore, suppressor mutations should identify interacting proteins in the SPB.

The suppressors of *kar1-Δ17* mapped to three linkage groups. The largest linkage group, comprising nine alleles, was allelic to *CDC31*. The second linkage group, *DSK2*, consisted of two alleles. The third linkage group consisted of two pseudoreversion alleles in *KAR1*.

One of the pseudorevertant *KAR1* mutations changed a novel residue created by the deletion junction in *kar1-Δ17*. Although this finding suggested that the temperature sensitivity of *kar1-Δ17* resulted from the novel junction, another deletion in this region with a different junction sequence, *kar1-Δ30*, also created a temperature sensitive phenotype. Therefore, it is unlikely that the specific junction sequences in *kar1-Δ17* are solely responsible for the temperature sensitive

phenotype. Furthermore, we have recently isolated several additional temperature sensitive *KAR1* alleles containing multiple point mutations in the SPB domain (E. A. VALLEN and M. D. ROSE, unpublished observations). Taken together, these data demonstrate that a variety of mutations within the SPB domain can create conditional alleles. Presumably the two pseudorevertants either stabilize the mutant protein or increase the affinity of a weak protein binding site.

CDC31 encodes a 161 amino acid protein that shares homology with the calcium-binding protein, calmodulin. There is 37% identity with yeast calmodulin (BAUM *et al.* 1986). Like *kar1-Δ17*, conditional *CDC31* alleles block SPB duplication and cells arrest with an enlarged single SPB, a large bud, a G2 content of DNA, and a single nucleus located at the bud neck (BYERS 1981; ROSE and FINK 1987). *CDC31* shares more homology (51% identity) with another calmodulin-like protein, caltractin/centrin, from the Chlamydomonas basal body (HUANG *et al.* 1988a,b; SALISBURY *et al.* 1988). Proteins antigenically related to caltractin/centrin have been found associated with the MTOC of higher eukaryotes (BARON and SALISBURY 1988; BARON *et al.* 1991; MOUDJOU *et al.* 1991). The phenotype of *cdc31* mutants and the localization of caltractin/centrin are consistent with the suggestion that Cdc31p is directly required for SPB duplication. The isolation of *CDC31* alleles that suppress mutations in *KAR1*, which encodes a protein associated with the SPB, provides strong genetic evidence that Cdc31p is a component of the SPB. Consistent with these findings, Cdc31p has recently been demonstrated to localize to the SPB in *S. cerevisiae* (SPRANG *et al.* 1993; S. BIGGINS and M. ROSE, manuscript in preparation).

Both alleles in the *DSK2* linkage group are strong suppressors of *kar1* mutations. Like the strongest $CDC31^{SUP}$ alleles, the *DSK2* mutations can suppress a complete deletion of *KAR1*. These mutations are unable to suppress a deletion of *CDC31* or the temperature sensitive defect of *CDC31-16* (our unpublished observations). We have not detected a phenotype caused by either of these alleles in a wild-type *KAR1* background or in a *kar1-Δ17* strain. Molecular cloning of the *DSK2* gene is in progress.

How does Cdc31p interact with Kar1p?

Several observations suggest that the suppression of *kar1-Δ17* by mutations in *CDC31* is closely related to the normal function of these two genes. First, the similarity of their mutant phenotypes strongly suggests that Kar1p and Cdc31p act at a similar or identical stage in SPB assembly. Second, high level expression of wild-type Cdc31p also suppresses *kar1-Δ17*. Therefore, a qualitative change of *CDC31* function is not required to suppress *kar1-Δ17*. Third, the reciprocal ability of *kar1* mutations to suppress the temperature sensitivity of *CDC31-16* suggests that *KAR1* is intimately involved with *CDC31*'s function. Fourth, hybrid Kar1p-β-galactosidase localization to the SPB is dependent on wild type *CDC31* (VALLEN *et al.* 1992a). Taken together, these observation suggest that Kar1p and Cdc31p act at a very closely associated, if not identical, step in SPB duplication.

The dominant $CDC31^{SUP}$ mutations do not lead to increased levels of Cdc31p (S. BIGGINS and M. ROSE, unpublished observations). Given the above evidence that the two proteins normally interact, it seems most likely that the dominant suppressor mutations act by enhancing a normal activity of Cdc31p, rather than by creating a novel activity.In principle, there are several possible models for the interaction between *CDC31* and *KAR1* during SPB duplication. One issue is whether the proteins interact directly or indirectly. For example, they may form a Cdc31p/Kar1p complex or be indirectly associated through a larger complex of SPB proteins. A second overlapping issue is whether the proteins act in one dependent pathway or in independent pathways leading to SPB duplication. For example, Kar1p might activate Cdc31p, which activates a downstream effector to initiate SPB duplication. Alternatively, both activation pathways might separately impinge on the SPB and be integrated to initiate SPB duplication.

The data presented in this report do not allow strong distinctions to be made between the different models. Data in support of a direct interaction include the allele specificity of the suppressors and the cosuppression between *kar1-Δ17* and *CDC31-16*. Superficially, the fact that the strongest $CDC31^{SUP}$ alleles can suppress a complete deletion of *KAR1* seems to argue against a direct interaction. However, this observation only establishes that the strongest suppressors do not act by increasing the affinity for binding between mutant Kar1p and Cdc31p. This observation also rules out any model in which Cdc31p must act before Kar1p in a dependent pathway. Instead the strong suppressors must act via the hyperactivation of Cdc31p or by enhancing its interaction with a downstream effector. If so, then the mechanism of the strong suppression may obscure other evidence of direct interaction.

Two pieces of evidence suggest that the suppressors do not simply bypass the requirement for Kar1p. First, several of the suppressors remain dependent upon *KAR1* function. Second the strong suppressors become supersensitive to *KAR1* gene expression and the sensitivity to Kar1p is directly correlated with the strength of the suppressor. It is difficult to rationalize these observations with a simple bypass mechanism in which the mutation has obviated the requirement for Kar1p. These observations seem more consistent with a model in which Kar1p and Cdc31p are cojointly required for SPB duplication.

One explicit model for the interaction between these two proteins that we favor is that Kar1p helps localize

Cdc31p to the SPB. Cdc31p would then initiate SPB duplication by interaction with a downstream effector. The *kar1-Δ17* mutation would disrupt partially the interaction between Karlp and Cdc31p, particularly at elevated temperatures. Accordingly, suppression may arise from several different mechanisms. Increased levels of wild-type Cdc31p should overcome the effects of the weakened interaction with mutant Karlp. Likewise, some of the $CDC31^{SUP}$ mutations might also restore binding to the mutant Karlp. Such suppressors would be allele specific and be unable to suppress a complete deletion of *KAR1*. Alternatively the weaker suppressors might be partially "hyperactivating" and remain dependent upon Karlp to reach a critical concentration at the SPB. Finally, as argued above, the strong $CDC31^{SUP}$ mutations must "hyperactivate" Cdc31p or strengthen Cdc31p's interaction with another component of the SPB. Such mutations would suppress a complete deletion of Karlp. Supersensitivity to Karlp might arise if excess "hyperactivated" Cdc31p at the SPB is toxic, due to premature assembly of SPB components or because the increased affinity for binding to the SPB blocks a later step in SPB assembly. That the $CDC31^{SUP}$ alleles cluster in the carboxy-terminal half of the protein suggests that this domain is essential for the interaction with the downstream effector or for its activation.

Taken together, our observations make Cdc31p a strong candidate for a protein that interacts with Karlp at the spindle pole body. Preliminary results suggest that Karlp and Cdc31p bind directly *in vitro* (S. BIGGINS and M. ROSE, manuscript in preparation). *DSK2* is a strong candidate for another protein that interacts with Cdc31p. Experiments are currently in progress to clone *DSK2* and identify additional proteins that interact with Cdc31p.

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LITERATURE CITED

ADAMS, A. E. M., and D. BOTSTEIN, 1989 Dominant suppressors of yeast actin mutations that are reciprocally suppressed. Genetics **121:** 675–683.

ADAMS, A. E. M., and J. R. PRINGLE, 1984 Relationship of actin and tubulin distribution to bud growth in wild-type and morphogenetic-mutant *Saccharomyces cerevisiae*. J. Cell Biol. **98:** 934–945.

BABU, Y. S., C. E. BUGG and W. J. COOK, 1988 Structure of calmodulin refined at 2.2 Å resolution. J. Mol. Biol. **204:** 191–204.

BARON, A. T., and J. L. SALISBURY, 1988 Identification and localization of a novel, cytoskeletal, centrosome-associated protein in PtK_2 cells. J. Cell Biol. **107:** 2669–2678.

BARON, A. T., T. M. GREENWOOD and J. L. SALISBURY, 1991 Localization of the centrin-related 165,000-M_r protein of PtK_2 cells during the cell cycle. Cell Motil. Cytoskeleton **18:** 1–14.

BAUM, P., C. FURLONG and B. BYERS, 1986 Yeast gene required for SPB duplication: homology of its product with Ca^{2+}-binding proteins. Proc. Natl. Acad. Sci. USA **83:** 5512–5516.

BOTSTEIN, D., and R. MAURER, 1982 Genetic approaches to the analysis of microbial development. Annu. Rev. Genetics **16:** 61–83.

BOYER, H. W., and D. ROULLAND-DUSSOIX, 1969 A complementation analysis of the restriction and modification of DNA in *Escherichia coli*. J. Mol. Biol. **41:** 459–472.

BULLOCK, W. O., J. M. FERNANDEZ and J. M. SHORT, 1987 XL1-Blue: A high efficiency plasmid transforming recA *Escherichia coli* strain with β-galactosidase selection. BioTechniques **5:** 376–379.

BYERS, B., 1981 Multiple roles of the spindle pole body in the life cycle of *Saccharomyces cerevisiae*, pp. 119–133 in *Molecular Genetics in Yeast*, edited by D. VON WETTSTEIN, J. FRIIS, M. KIELLAND-BRANDT and A. STENDERUP. Munksgaard, Copenhagen.

BYERS, B., and L. GOETSCH, 1991 Preparation of yeast cells for thin-section electron microscopy. Methods Enzymol. **194:** 602–608.

CARLSON, M., and D. BOTSTEIN, 1982 Two differentially regulated mRNA's with different 5′ ends encode secreted and intracellular forms of yeast invertase. Cell **28:** 145–154.

CONDE, J., and G. R. FINK, 1976 A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. USA **73:** 3651–3655.

DAVIS, R. W., D. BOTSTEIN and J. R. ROTH, 1980 *Advanced Bacterial Genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

DAVIS, T. D., M. S. URDEA, F. R. MASIARZ and J. THORNER, 1986 Isolation of the yeast calmodulin gene: calmodulin is an essential protein. Cell **47:** 423–431.

FINK, G. R., and J. CONDE, 1976 Studies on *KAR1*, a gene required for nuclear fusion in yeast, pp. 414–419 in *International Cell Biology 1976–1977*, edited by B. R. BRINKLEY and K. R. PORTER. The Rockefeller University Press, New York.

HARTWELL, L. H., 1967 Macromolecular synthesis in temperature sensitive mutants of yeast. J. Bacteriol. **93:** 1662–1670.

HOFFMAN, C. S., and F. WINSTON, 1987 A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *E. coli*. Gene **57:** 267–272.

HUANG, B., D. M. WATTERSON, V. D. LEE and M. J. SCHIBLER, 1988a Purification and characterization of a basal body-associated Ca^{2+}-binding protein. J. Cell Biol. **107:** 121–131.

HUANG, B., A. MENGERSEN and V. D. LEE, 1988b Molecular cloning of cDNA for caltractin, a basal body-associated Ca^{2+}-binding protein: homology in its protein sequence with calmodulin and the yeast *CDC31* gene product. J. Cell Biol. **107:** 133–140.

IKURA, M., S. SPERA, G. BARBATO, L. E. KAY, M. KRINKS *et al.*, 1991 Secondary structure and side-chain ^1H and ^{13}C resonance assignments of calmodulin in solution by heteronuclear multidimensional NMR spectroscopy. Biochemistry **30:** 9216–9228.

JOSHI, H. C., M. J. PALACIOS, L. MCNAMARA and D. W. CLEVELAND, 1992 γ-Tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. Nature **356:** 80–83.

KILMARTIN, J. V., and A. E. M. ADAMS, 1984 Structural rearrangements of tubulin and actin during the cell cycle of the yeast *Saccharomyces*. J. Cell Biol. **98:** 922–933.

KIM, J., P. O. LJUNGDAHL and G. R. FINK, 1990 *kem* mutations affect nuclear fusion in *Saccharomyces cerevisiae*. Genetics **126:** 799–812.

KLEE, C. B., T. H. CROUCH and P. G. RICHMAN, 1980 Calmodulin. Annu. Rev. Biochem. **49:** 489–515.

MOUDJOU, M., M. PAINTRAND, B. VIGUES and M. BORNENS, 1991 A human centrosomal protein is immunologically related to basal body-associated proteins from lower eukaryotes and is involved in the nucleation of microtubules. J. Cell Biol. **115:** 129–140.

OAKLEY, B. R., C. E. OAKLEY, Y. YOON and M. K. JUNG, 1990 γ-Tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. Cell **61:** 1289–1301.

OAKLEY, C. E., and B. R. OAKLEY, 1989 Identification of γ-tubulin, a new member of the tubulin superfamily encoded by *mipA* gene of *Aspergillus nidulans*. Nature **338:** 662–664.

ORR-WEAVER, T. L., J. W. SZOSTAK and R. J. ROTHSTEIN, 1983 Genetic applications of yeast transformation with linear and gapped plasmids. Methods Enzymol. **101:** 228–245.

PERKINS, D. D., 1949 Biochemical mutants of the smut fungus *Ustilago maydis*. Genetics **34:** 607–629

ROSE, M. D., and G. R. FINK, 1987 *KAR1*, a gene required for function

of both intranuclear and extranuclear microtubules in yeast. Cell **48:** 1047–1060.

ROSE, M. D., F. WINSTON and P. HIETER, 1990 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

SALISBURY, J. L., A. T. BARON and M. A. SANDERS, 1988 The centrin-based cytoskeleton of *Chlamydomonas reinhardtii*: distribution in interphase and mitotic cells. J. Cell Biol. **107:** 635–641.

SANGER, F., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SIKORSKI, R. S., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

SOLOMON, F., 1991 Analyses of the cytoskeleton in *Saccharomyces cerevisiae.* Annu. Rev. Cell Biol. **7:** 633–662.

SPRANG, A., I. COURTNEY, U. FACKLER, M. MATZNER and E. SCHIEBEL, 1993 The calcium-binding protein cell division cycle 31 of *Saccharomyces cerevisiae* is a component of the half bridge of the spindle pole body. J. Cell Biol. **123:** 405–416.

STEARNS, T., L. EVANS and M. KIRSCHNER, 1991 γ-tubulin is a highly conserved component of the centrosome. Cell **65:** 825–836.

STRYNADKA, N. C. J., and M. N. G. JAMES, 1989 Crystal structures of the helix-loop-helix calcium-binding proteins. Annu. Rev. Biochem. **58:** 951–998.

VALLEN, E. A., 1992 Genetic Analysis of the yeast microtubule organizing center, or, how a used *KAR* breaks down. Ph.D. Thesis, Princeton University, Princeton, N.J.

VALLEN, E. A., T. Y. SCHERSON, T. ROBERTS, K. VAN ZEE and M. D. ROSE, 1992a Asymmetric mitotic segregation of the yeast spindle pole body. Cell **69:** 505–515.

VALLEN, E. A., M. A. HILLER, T. Y. SCHERSON and M. D. ROSE, 1992b Separate domains of *KAR1* mediate distinct functions in mitosis and nuclear fusion. J. Cell Biol. **117:** 1277–1287.

WEIL, C. F., C. E. OAKLEY and B. R. OAKLEY, 1986 Isolation of *mip* (microtubule-interacting protein) mutations of *Aspergillus nidulans.* Mol. Cell. Biol. **6:** 2963–2968.

WINEY, M., and B. BYERS, 1992 The spindle pole body of *Saccharomyces cerevisiae*: a model for genetic analysis of the centrosome cycle, pp. 201–222 in *The Centrosome*, edited by V. KALNINS. Academic Press, Orlando, Fla.

WINEY, M., L. GOETSCH, P. BAUM and B. BYERS, 1991 *MPS1* and *MPS2*: novel yeast genes defining distinct steps of spindle pole body duplication. J. Cell Biol. **114:** 745–754.

ZHENG, Y., M. K. JUNG and B. R. OAKLEY, 1991 γ-tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. Cell **65:** 817–823.

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